Indiana State Department of Health

August 2016

Dear Healthcare Provider:

The purpose of this letter is to inform you that the Indiana State Department of Health Laboratories is now offering the Trioplex rRT-PCR assay, a molecular test for Zika, Dengue, and Chikungunya viruses. The Trioplex rRT-PCR assay has been authorized for the qualitative detection and differentiation of Dengue and Chikungunya viruses from serum and cerebrospinal fluid (CSF) or for Zika virus from serum, urine, CSF, and amniotic fluid. Submissions of CSF, urine, or amniotic fluid for Zika testing should be paired with a serum specimen from the same patient.

Patient screening and case approval will continue as per our previous guidance, and based on epidemiological data (i.e. travel to an endemic are and exhibiting symptoms or women who are asymptomatic but pregnant). Please follow these steps for case approval prior to submitting specimens:

1. Navigate to http://www.in.gov/isdh and click the banner at the top that says "Zika Virus."
2. This will take you to the main Zika page. Click the link that says "For Providers".
3. This link will take you to the indications and instructions for Zika virus testing. Providers should follow the listed steps, which include reviewing if the patient meets Zika testing criteria, and faxing the "ISDH Zika Virus Authorization Form" to 317-234-2812.
4. You will receive a response within one (1) business day.

Please note that a negative Zika virus rRT-PCR does not always rule out Zika virus infection. During the first 14 days of symptom onset, Zika RNA may be identified in serum, and rRT-PCR is the preferred test. However, Zika RNA decreases over time, and a negative rRT-PCR does not preclude Zika virus infection. This is why paired serology will be performed with all submissions at a secondary facility. Zika virus RNA may be identified in urine for a longer period of time than in serum. It is for this reason that paired serum and urine are requested when a patient's symptom onset is less than 14 days prior to specimen collection. [See diagram of testing process below.]

Due to the emerging and urgent need for enhanced Zika virus diagnostics, the Trioplex rRT-PCR Assay was made available by an Emergency Use Authorization (EUA) from the FDA, and is exempt from some of the regulations and validations of other assays. Information sheets regarding the EUA of the Trioplex rRT-PCR will accompany the final result report. These

information sheets are tailored for three main audiences: health care providers, pregnant women, and other patients. Should you not receive these information sheets please contact the laboratory immediately at 317-921-5500.

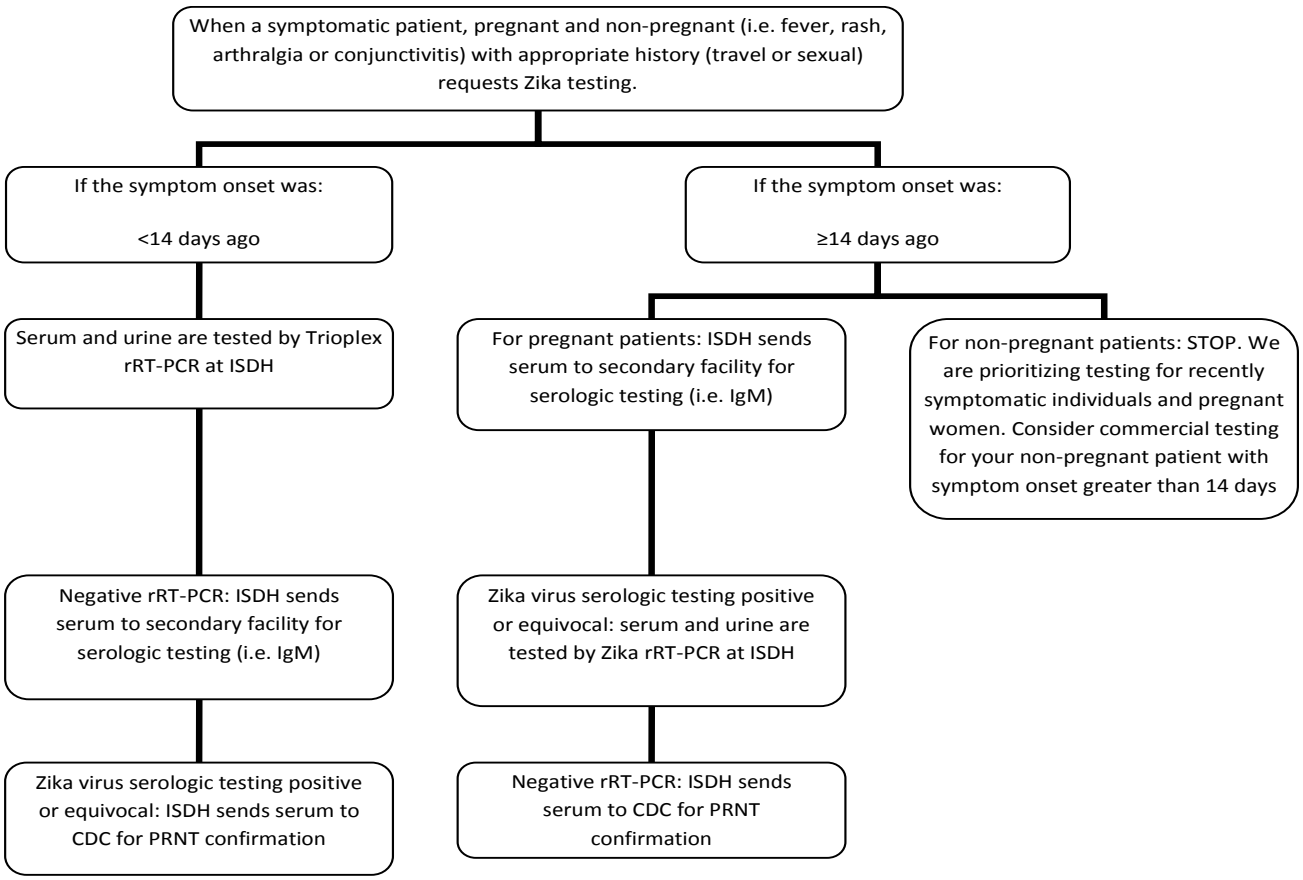
Attached to this letter you will find specimen submission, collection, packaging, and shipping information. Please read and follow these directions carefully. We have also included test interpretation guidelines for your benefit.

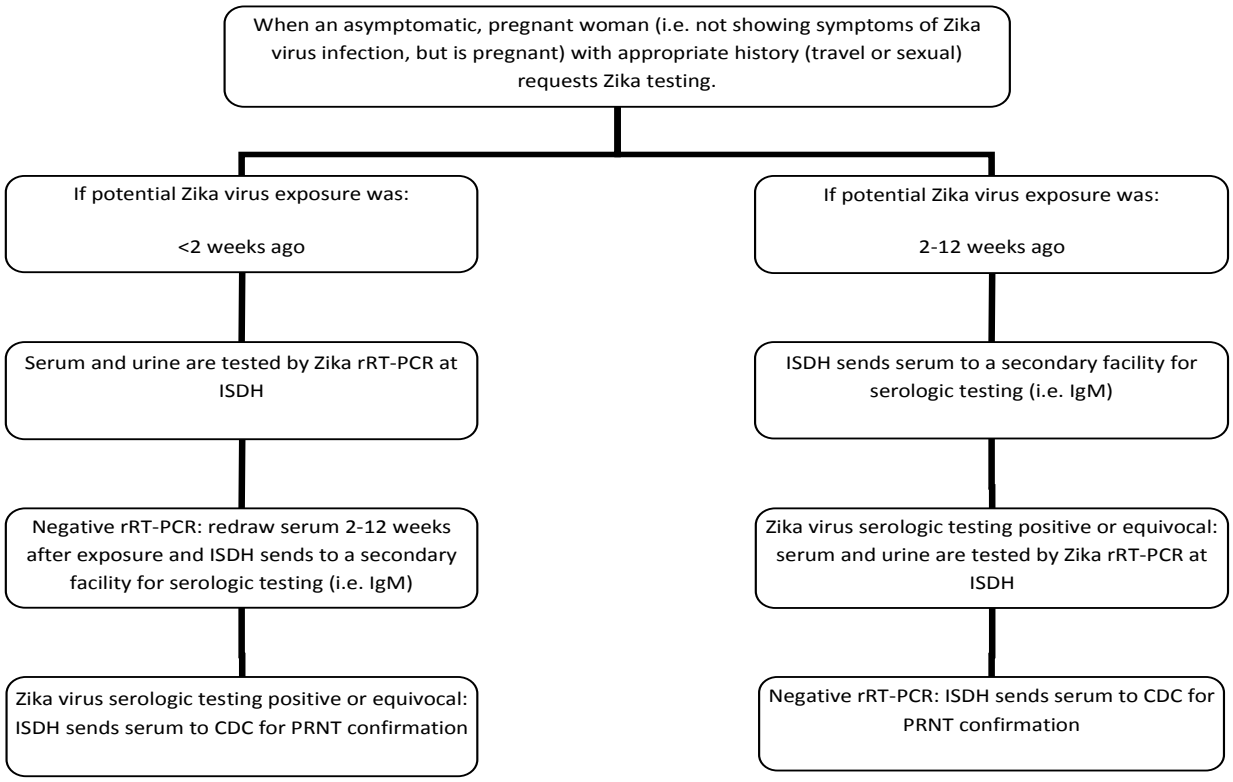
Sincerely,

Judith Lovchik, PhD, D(ABMM)

Assistant Commissioner, Laboratory Services
Indiana State Department of Health

Indiana State Department of Health Zika Testing Process





Indiana State Department of Health

Zika Virus Specimen Collection and Transport Guidelines

For Providers or Laboratories Shipping their own Specimens
Whole Blood/Serum and Urine

Specimens:

Exposed symptomatic persons (pregnant and non-pregnant)

- If symptom onset is less than 14 days prior to specimen collection, both a serum and a urine specimen are requested.
- If symptom onset is more than 14 days prior to collection, only a serum specimen is requested.

Asymptomatic pregnant women

- If potential Zika virus exposure is less than 2 weeks prior to specimen collection, both a serum and a urine specimen are requested.
- If potential Zika virus exposure is more than 2 weeks prior to specimen collection, only a serum specimen is requested.

<u>For serum specimens:</u>
Submit at least 3 mL of serum in a screw-capped tube. Alternatively, collect at least 10 mL of whole blood in a serum separator or red-top tube (serum separators include tiger top and gold-top tubes). Label the specimen tube with the patient's name (first and last), date of birth, specimen type, and date of collection. Whole blood specimens collected in serum separator tubes may be centrifuged prior to shipping. Hemolyzed specimens will be rejected.

<u>For urine specimens:</u>
Submit 1-2 mL of urine in a screw-capped or conical tube (sterile container without preservative). Please do not submit larger volumes of urine. Label the specimen tube with the patient's name (first and last), date of birth, specimen type, and date of collection. Specimens that leak in transit will be rejected.

Storage:

Once collected, place the specimens at 2-8°C until ready to ship. Hemolyzed serum specimens, or specimens that leak in transit will not be accepted or tested. Specimens should be shipped to the ISDH Laboratories on cold packs within 24-48 hours from the time of collection. If a specimen is collected on the day prior to a weekend or holiday, please keep the specimen at 2-8°C and ship on the next available business day.

Paperwork:
In LimsNet, complete the "Arbovirus (human)" submission form, ensuring that the patient information on the collection tube matches the information on the form. One form is required per specimen. **Specimens are required to be submitted through LimsNet.** If you are unfamiliar with LimsNet, refer to the following LimsNet Quick Start Guide for information.

Please fill out as much patient information as possible. **Patient history, including symptoms, date of symptom onset, exposure history, and travel history (including dates and countries of travel) are required for specimen submission.** Once complete, print the LimsNet coversheet. Include the LimsNet coversheet with the specimen submission. Specimens received without this document will not be tested.

Shipping:
Specimens should be shipped Category B (UN3373 Biological Substances) *on cold packs*. Wrap the labeled specimen tube with absorbent material and place in a watertight secondary container. Place the watertight secondary container in a rigid outer container. Place the paperwork in the outer container or in a plastic bag before placing in the shipping container. See the Category B Packaging & Shipping Instructions for more details.

Specimens should be shipped to arrive at ISDH Laboratories Monday through Friday.

Our address:

Indiana State Department of Health Laboratories
Attention: Virology Laboratory
550 W. 16th Street, Suite B
Indianapolis, IN 46202

For questions or comments regarding specimen collection, storage, or transport, please contact:

Brian Pope (317) 921-5843

Indiana State Department of Health

Zika Virus Specimen Collection and Transport Guidelines

For Providers Shipping through another Laboratory

Whole Blood/Serum and Urine

Specimens:

Exposed symptomatic persons (pregnant and non-pregnant)

- If symptom onset is less than 14 days prior to specimen collection, both a serum and a urine specimen are requested.
- If symptom onset is more than 14 days prior to collection, only a serum specimen is requested.

Asymptomatic pregnant women

- If potential Zika virus exposure is less than 2 weeks prior to specimen collection, both a serum and a urine specimen are requested.
- If potential Zika virus exposure is more than 2 weeks prior to specimen collection, only a serum specimen is requested.

<u>For serum specimens:</u>
Submit at least 3 mL of serum in a screw-capped tube. Alternatively, collect at least 10 mL of whole blood in a serum separator or red-top tube (serum separators include tiger top and gold-top tubes). Label the specimen tube with the patient's name (first and last), date of birth, specimen type, and date of collection. Whole blood specimens collected in serum separator tubes may be centrifuged prior to shipping. Hemolyzed specimens will be rejected.

<u>For urine specimens:</u>
Submit 1-2 mL of urine in a screw-capped or conical tube (sterile container without preservative). Please do not submit larger volumes of urine. Label the specimen tube with the patient's name (first and last), date of birth, specimen type, and date of collection. Specimens that leak in transit will be rejected.

Storage:
Once collected, place the specimens at 2-8°C until ready to ship. Hemolyzed serum specimens, or specimens that leak in transit will not be accepted or tested. Specimens should be shipped to the ISDH Laboratories on cold packs within 24-48 hours from the time of collection. If a specimen is collected on the day prior to a weekend or holiday, please keep the specimen at 2-8°C and ship on the next available business day.

Paperwork:
Complete the paper copy of the "Arbovirus (human)" submission form, ensuring that the patient information on the collection tube matches the information on the form. One form is required per specimen. **Specimens are required to be submitted through LimsNet. Please ensure that your laboratory is submitting the test requisition in LimsNet. Reporting of results will be delayed until the test request is made in LimsNet.** If your laboratory is unfamiliar with LimsNet, have them refer to the following LimsNet Quick Start Guide for information.

Please fill out as much patient information as possible. **Patient history, including symptoms, date of symptom onset, exposure history, and travel history (including dates and countries of travel) are required for specimen submission.**

Shipping:
Specimens should be shipped Category B (UN3373 Biological Substances) *on cold packs*. Wrap the labeled specimen tube with absorbent material and place in a watertight secondary container. Place the watertight secondary container in a rigid outer container. Place the paperwork in the outer container or in a plastic bag before placing in the shipping container. See the Category B Packaging & Shipping Instructions for more details.

Specimens should be shipped to arrive at ISDH Laboratories Monday through Friday.

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Brian Pope (317) 921-5843

Table 1. Interpretation of Trioplex Assay results (serum).

Interpretation			**Conclusion**	**Additional Notes:**
Negative			Zika, Dengue, or Chikungunya RNA not detected.	Serology recommended.
Inconclusive			Specimen inconclusive for the presence of Zika, Dengue, and Chikungunya RNA. An inconclusive result may occur in the case of an inadequate specimen.	Collect another specimen and resubmit.
Positive			Key: + indicates that the Trioplex assay was positive for that marker, - indicates that the Trioplex assay was negative for that marker; DENV – Dengue virus, CHIKV – Chikungunya virus, ZIKA – Zika virus	
DENV	CHIKV	ZIKA		
+	-	-	Dengue RNA detected. Chikungunya or Zika RNA not detected.	Dengue virus confirmed.
-	+	-	Chikungunya RNA detected. Dengue or Zika RNA not detected.	Chikungunya virus confirmed.
-	-	+	Zika RNA detected. Chikungunya or Dengue RNA not detected.	Zika virus confirmed.
+	+	-	Dengue and Chikungunya RNA detected. Zika RNA not detected.	Dengue and Chikungunya viruses confirmed.
+	-	+	Dengue and Zika RNA detected. Chikungunya RNA not detected.	Dengue and Zika viruses confirmed.
-	+	+	Zika and Chikungunya RNA detected. Dengue RNA not detected.	Zika and Chikungunya viruses confirmed.
+	+	+	Dengue, Chikungunya, and Zika RNA detected.	Dengue, Chikungunya, and Zika viruses confirmed.

Table 2. Interpretation of Trioplex Assay results (urine).

Interpretation	Conclusion	Additional Notes:
Negative	Zika RNA not detected.	Serology recommended. Serology is only performed on serum specimens.
Inconclusive	Specimen inconclusive for the presence of Zika RNA. An inconclusive result may occur in the case of an inadequate specimen.	Collect another specimen and resubmit.
Positive	Zika RNA detected.	Zika virus confirmed.

Category B Packaging & Shipping Instructions

There are five (5) main components to packaging and shipping Category B:

1. Primary Receptacle
2. Absorbent Material
3. Secondary Receptacle
4. Outer Packaging
5. Package Markings

Primary Receptacle:
The specimen is placed directly inside the primary receptacle, so it is important that this receptacle is leak-proof. Primary receptacles must have positive closures, such as a screw-cap, snap-on, or push-on lids. Secure the lid to the canister with adhesive tape or Parafilm.
Both primary and secondary receptacles should be able to withstand an internal pressure producing a pressure differential of not less than 95 kPa (14 psi) in the range of -40°C to 55°C (-40°F to 130°F).

Absorbent Material
An absorbent material should be wrapped around the primary receptacle. This absorbent material should be capable of absorbing the entire volume of the primary receptacle in the event that it was broken in transit. More than one primary receptacle may be included in a single shipment; each should be individually wrapped in the absorbent material to prevent contact. Examples of appropriate absorbent materials include paper towels, cotton balls, or cellulose wadding.

Secondary Receptacle
More than one primary receptacle may be included in a single secondary container if individually wrapped in absorbent material. Do not over-pack the secondary receptacle. Secondary receptacles, such as a sealed plastic bag, a plastic or aluminum screw-cap canister, or a sealed Styrofoam container (at least 1" thick), must also be leak-proof. *Place the secondary receptacle into an insulated shipping container with cold packs to keep the specimen cool while shipping.*

Outer Packaging
The secondary receptacle and insulated shipping container should be placed into a study exterior package before shipping. Acceptable exterior packing materials include corrugated fiberboard, wood, metal, or rigid plastic. Styrofoam boxes without an external fiberboard backing, plastic bags, paper envelopes, or boxes with extra external markings are not considered appropriate exterior packaging materials.

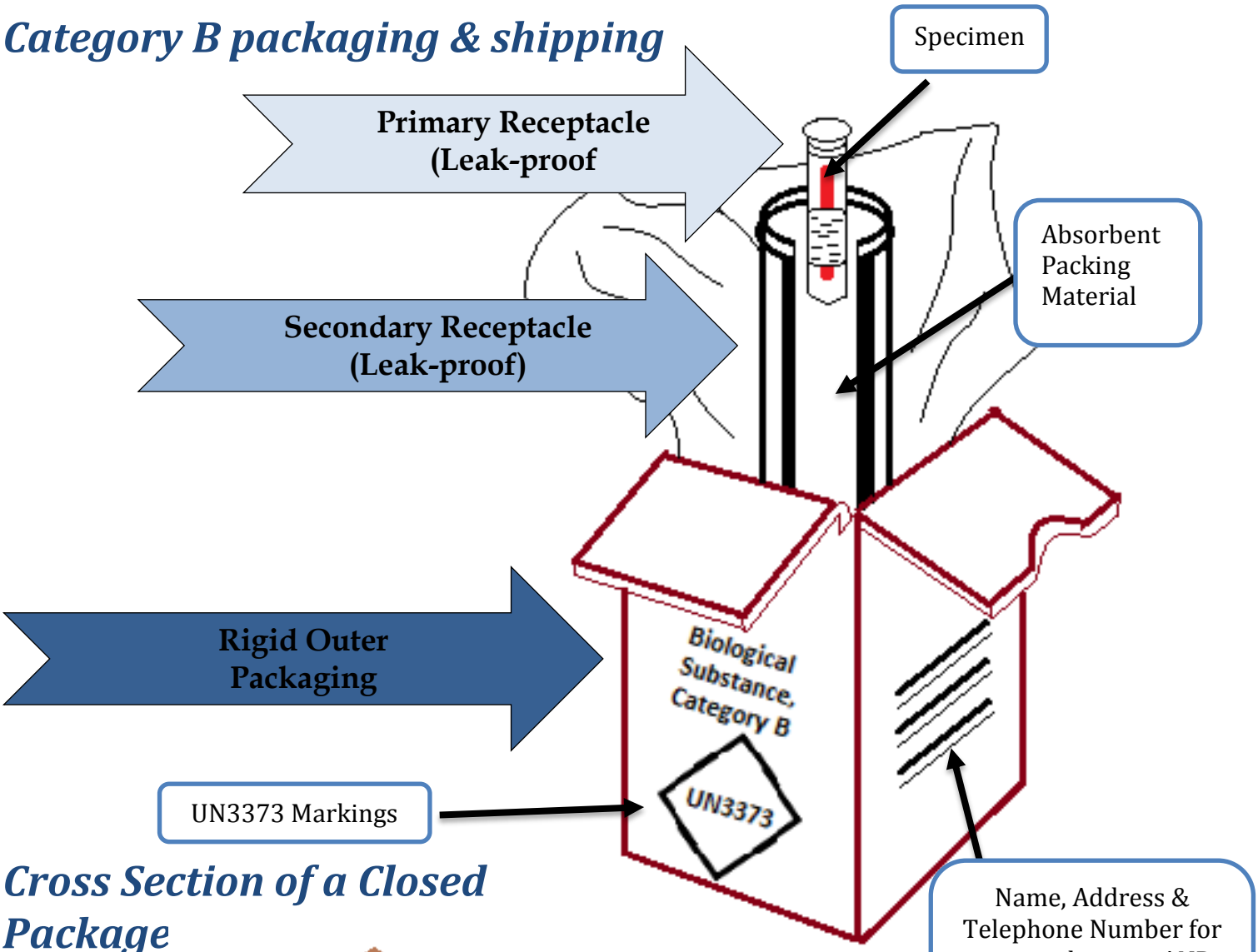
Markings
Four main items must be present on the exterior of each Category B package: (1) the UN3373 diamond (see diagram below); (2) the words "Biological Substance Category B" at least 6 mm tall next to the UN3373 symbol; (3) the name, address, and telephone number of the shipper; (4) the name, address, and telephone number of the receiver.

Paperwork should be included in each shipment between the secondary receptacle and the sturdy exterior packaging. Specimens that arrive at ISDH without this paperwork will not be tested. Please place the paperwork in the package in such a way that it does not get wet during shipping.

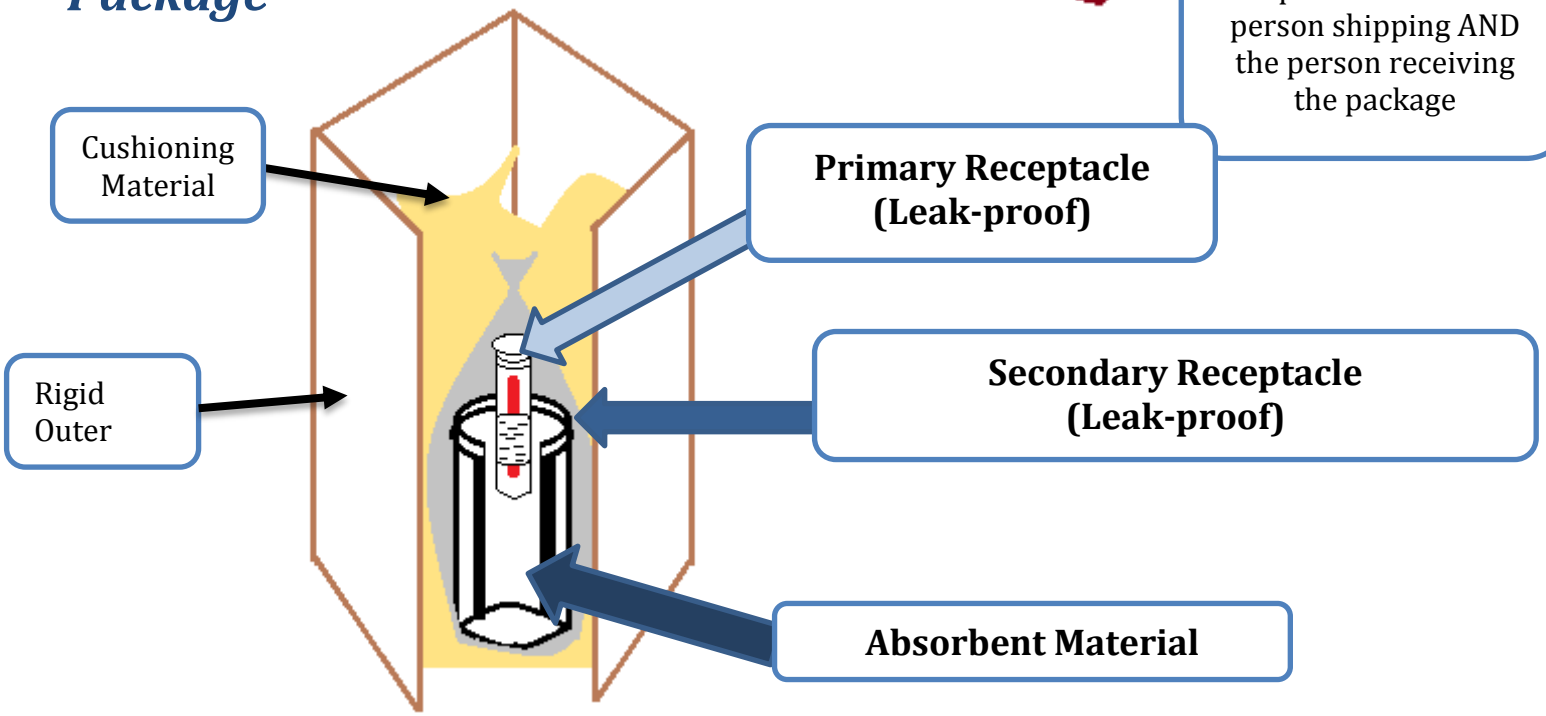
1. ***LimsNet coversheet:*** *one per specimen*

For questions regarding Category B Packaging & Shipping, please call ISDH at (317) 921-5500.

Category B packaging & shipping



Cross Section of a Closed Package



Indiana State Department of Health

LimsNet Quick Start Guide

LimsNet is the ISDH Laboratories specimen submission portal. LimsNet allows the user to submit specimens and receive results electronically.

Getting started

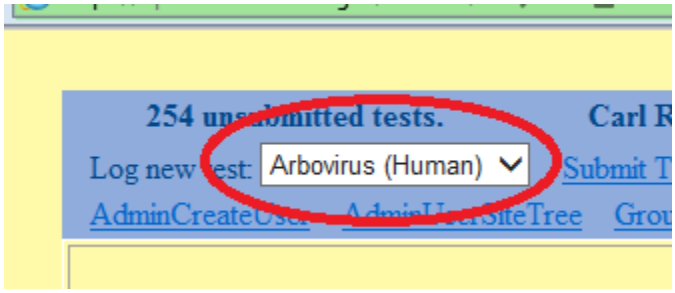
Contact LimsNet Support at 888-535-0011 or email at LimsAppSupport@isdh.in.gov. We will create an account for your organization and any users that you wish have access to this account.

The LimsNet home page is: http://limsnet.isdh.in.gov

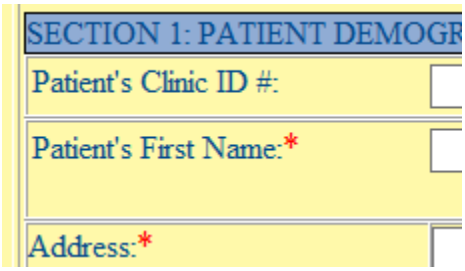
Be sure to use Internet Explorer to access LimsNet. Other browsers are not supported.

Submitting a Specimen

To begin, choose "Arbovirus (Human)" by clicking the arrow next to "Log new test" in the navigation bar:



You will now be directed to the "Arbovirus (Human)" submission form. Use this form to submit test requests for Zika, Chikungunya, or Dengue testing.



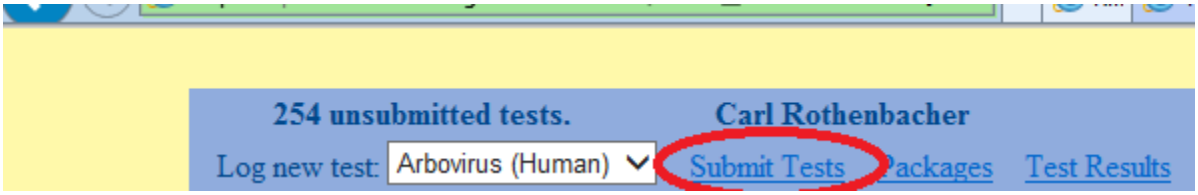
Next, fill out the patient and specimen information. Any fields marked with an asterisk (*) are required for specimens submission.

When finished, press Save at the bottom of the form. If there are errors or missing information, LimsNet will alert you before your data can be saved.

Sending the sample to ISDH

The next step is to ship your sample to the ISDH Laboratories. For information about packaging and shipping your specimen, see the Category B Packaging & Shipping Instructions document included in your submission package.

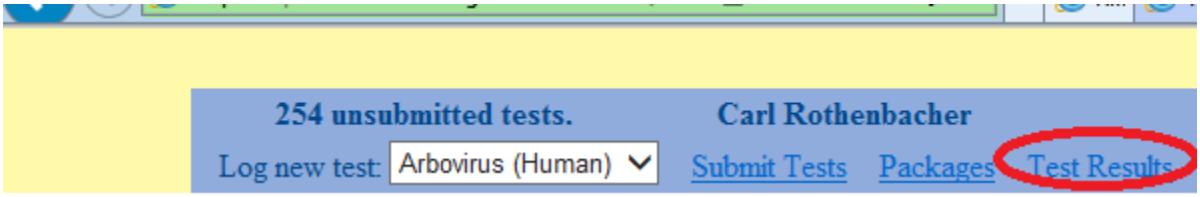
When your package is ready to ship, click on "Submit Tests" in the Navigation bar.



On the next screen, select the sample you wish to ship and click "Mark as Shipped" at the bottom of the screen. LimsNet will then display a barcoded cover page. Print this cover page and include it in the package containing your samples.

Viewing Test Results

To check the status of your testing request, or to view a report, click on "Test Results" in the navigation bar.



The test results screen allows you to see the status of each sample you have logged into LimsNet. A specimen status may be: Unshipped, In Transit, Received, Pending, Preliminary, and Released.

Any sample with the status of "Preliminary" or "Released" has a results report that can be viewed by pressing the "View Report" button. Pressing this button will display a PDF containing the ISDH Laboratories report for the selected sample.

For More Information

The LimsNet home page has both a downloadable manual and an instructional slide show in PDF format. These documents go into further detail than what is presented in this Quick Start Guide.

As always, feel free to contact us on the LIMS App Support team with any questions.

Version 08/09/16

Frequently Asked Questions - Trioplex RT-PCR Assay

1. **What are specimen types appropriate for Zika Virus Testing?**

   Preferred specimen types: Serum or Whole Blood *and* Urine
   Please see the ISDH Zika Virus Specimen Collection and Transport Guidelines for more information.

2. **Will approved specimens be tested for other arboviruses (i.e. Dengue or Chikungunya) or just Zika?**

   Yes, serum specimens from symptomatic patients that are collected within 14 days of symptom onset will be tested for both Dengue and Chikungunya, as these viruses share similar symptoms and mosquito vectors. However, a negative Trioplex RT-PCR result does not rule out the possibility of infection with Dengue or Chikungunya. Dengue and Chikungunya are not validated on urine specimens. Please ensure a serum specimen is submitted with every urine!

3. **How long after symptom onset can a specimen be collected/tested?**

   For PCR: up to 14 days

   For Serology: up to 12 weeks

4. **What is Category B shipping?**

   Please see the Category B Packaging & Shipping Instructions document for more information.

5. **Can't I just stick the specimen in a box and send it to you (i.e. not Cat B)?**

   No, shipping improperly packaged goods could result in hefty fines for you and your institution from the Department of Transportation. Improperly packaged biological specimens are a safety risk for postal service and laboratory personnel.

6. **Can you send me shipping or specimen collection materials?**

   Unfortunately no, ISDH cannot provide shipping or specimen collection materials at this time. We do, however, provide this testing at no cost to you.

7. **Can't I just send this directly to the CDC?**

   CDC has requested that states assist in triaging the large quantity of Zika testing requests. Because of this, only specimens that arrive from the Indiana State Department of Health are currently being accepted for Indiana patients.

8. **Can I send this to another commercial lab?**

   Certainly! Each clinician must decide what is best for their patient. We want you to have as much information at your fingertips, however, to help you make your Zika testing decisions.

There are currently two commercially available tests that have been approved by the FDA for Zika PCR. These methods only detect Zika, and do not test for Dengue or Chikungunya, two viruses that share similar symptoms and mosquito vectors as Zika.

If a patient with an appropriate travel history has been symptomatic <3 days, PCR is usually sufficient, and may be performed at ISDH or elsewhere if desired. If, however, the patient has been symptomatic from 3-14 days, or the PCR is negative, serology is also strongly recommended. Therefore, we request that patients who are in this 3-14 day time period, or whose PCR results were negative, have a second specimen submitted to ISDH for serology. Any positives diagnosed outside of ISDH should submit via the same process your facility uses when reporting lab results for reportable conditions, which may include contacting your hospital/facility ICP.

One further consideration is that many of the commercially available Zika PCR tests are only approved for testing with serum specimens. The CDC has recently indicated that urine is the best specimen for the detection of Zika by PCR. Here at ISDH, we are currently requesting paired serum and urine for all Zika testing requests.

Laboratory findings that are positive for an Arboviral disease are required to be reported immediately to ISDH by the Indiana Communicable Disease Rule 410 IAC 1-2.5.-82. This reporting process should be the same one that your facility uses when reporting lab results for reportable conditions, which may include contacting your hospital/facility ICP.

9. What is the turn-around-time for this test?

For specimens tested at ISDH Laboratories by the Trioplex RT-PCR assay:

2-3 business days from the time a specimen is received

For specimens tested by Serology:

2-3 weeks from the time a specimen is received

10. For patients that screen negative (IgM negative) is collection of a convalescent specimen still required?

No, convalescent specimens are no longer being accepted. No additional convalescent testing is being performed at this time.

11. I have a patient in the office today (Friday), can I collect the specimens today, or do I need to wait until Monday?

Please collect and place the specimens in the refrigerator over the weekend and ship to us first thing on Monday. Please do not freeze the specimens.

12. I have a patient in the office today that has not yet been approved for testing. Can I collect specimens today while they're in the office?

Yes, you may collect the specimen first and receive approval second. However, please note that specimens collected from patients who does not ultimately receive authorization will not be tested. To review the criteria for test approval, and compare your patient's exposure and travel history with the Authorization of Specimens for Zika Virus (ZIKV) Testing flowchart:

- Navigate to http://www.in.gov/isdh and click the banner at the top that says "Zika Virus."
- This will take you to the main Zika page. Click the link that says "For Providers".
- This link will take you to the indications and instructions for Zika virus testing. Providers should follow the listed steps, which include reviewing if the patient meets Zika testing criteria, and faxing the "ISDH Zika Virus Authorization Form" to 317-234-2812.
- You will receive a response within 1 business day.